

# Airborne Bacteria in Dairy Farms

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The environmental quality of farms plays an important role in the food safety of the dairy industry because it may influence the microbial communities in milk. The microorganisms present in the different areas of a farm have an influence on this environmental quality, using the air as a vehicle of dissemination. However, the ability of this airborne microbial community to contaminate the milk, like the sources of origin of these microorganisms, has not been well studied in dairy farms until now.

Keywords: dairy farm environment ; air quality ; airborne microorganism ; milk

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## 1. Introduction

Due to the beneficial properties of milk and the wide range of essential nutrients it provides, there are more than 6 billion consumers of milk and milk products throughout the world, the majority of them in developing countries <sup>[1]</sup>. Throughout the twentieth century, milk became a raw material of an important industry and was made available to consumers in a comfortable, safe, and economic way. Nowadays, milk production is a dynamic and growing industry that is essential to the economies of many countries. Approximately 150 million family farms around the globe are engaged in milk production <sup>[2]</sup>. The farmgate milk price depends on the milk's compositional quality, but the hygienic quality of the milk is also very important.

The control of milk quality is one of the most important problems in the dairy sector. Although traditionally the quality of the milk was evaluated according to its composition, due to the high levels of milk production at the end of the last century, quality control systems are now based on health and hygienic parameters too, specifically on the total mesophilic bacteria and the somatic cell count in milk from bulk tanks <sup>[3]</sup>. Laws on food security and nutrition, such as Regulation EC n° 853/2004 <sup>[4]</sup> in Europe, usually establish the boundaries for these parameters.

The presence of microorganisms in milk can have their origin in intramammary infections (affecting somatic cell count) and/or environmental contamination (affecting total aerobic microbial count), such as udder hygiene, milking parlor, milk houses, etc. <sup>[5]</sup>. Gonzalo et al. <sup>[6]</sup> pointed out that the total bacteria counts allow the evaluation of the level of adequacy of cleaning practices on the dairy farm, in addition to ensuring the correct quality of the milk and milk products that derive from it. However, this count is less representative for the evaluation of the microbial quality of milk, and that is why several studies have assessed other bacterial groups of milk in cattle <sup>[7]</sup>, in goats <sup>[8]</sup>, and in sheep <sup>[9]</sup>.

Milk microbiota has a great influence on the safety and quality of dairy products, carrying out various functions such as facilitating dairy fermentations or causing spoilage or disease, among others <sup>[10]</sup>. For this reason, it becomes very important to know the variety of routes that these microorganisms could use to reach the milk. Good veterinary practices can be implemented, as well as good management and hygiene of the milking parlor, which can avoid direct contamination of the milk <sup>[11]</sup>. However, it is not so easy to control the environmental quality of dairy farms.

In recent years, the dairy farm environment has become a focus of interest in the agro-food industry. This is due to the effects of the diversity of activities carried out on the farms, which could affect the final product, the milk, and, therefore, could have social and economic impacts on the production of both milk and dairy products (mainly cheese).

## 2. Dairy Farm Environment

Dairy farms have a dynamic environment with associated microbial communities, which have natural reservoirs among components of this ecosystem <sup>[12]</sup>. This environment is very complex due to the microbial diversity present in the different areas of the farm (such as the milking parlor, the stables, and even the environment surrounding the farm), and it does not guarantee good application of the Hazard Analysis Critical Control Points system. For this reason, the health authorities

recommend the application of good manufacturing practices on the farm, as the European legislation has done (Regulation EC n° 178/2002 <sup>[13]</sup> and Regulation EC n° 853/2004 <sup>[4]</sup>).

Adequate sanitary environmental conditions in food processing plants could reduce the level of the microbial communities on the farm. However, it is impossible to keep environmental contamination, such as bacteria, yeasts, and molds, at a minimum level in the environment of food processing <sup>[14]</sup>. This is mainly due to the air present in the environment, which is the transport vehicle of matter particles and bioaerosols. For this reason, it is important to pay special attention to the quality of this air, as one of the main routes of contamination of dairy farms. The air is a hostile environment as a habitat for microorganisms because they are stressed by the lack of nutrients and dehydration, but it is an important vehicle for dissemination since we can find all kinds of bacteria in it <sup>[15]</sup>. The microorganisms could travel through the air adhered to dust particles or droplets, or even as single particles, until they fall and are deposited on the surrounding surfaces <sup>[16]</sup>. The air, as a source of contamination through dissemination, has been properly researched in numerous studies of food processing plants <sup>[14]</sup> as well as in the field of enology <sup>[17]</sup>.

Several studies related to environmental contamination on dairy farms have focused on pathogens and decomposition bacteria. As far as is known, few studies have focused on environmental microbiology, from the point of view of contamination and its effect on milk and dairy products <sup>[18]</sup>. It is now clear that the microbiological load present in the environment of farms could have a positive influence on milk, inasmuch as the possible existence of some airborne microorganisms, such as lactic acid bacteria, would permit an enrichment of the nutritional and organoleptic qualities of the milk, as has been previously studied in wineries <sup>[17]</sup>. In addition, the microbiota naturally present in milk may have a protective effect against the growth of pathogens found in the air <sup>[19]</sup>. However, the ability of the airborne microbial community to contaminate milk remains an ongoing concern, and the sources of the origin of contamination of those microorganisms that might affect the natural microbiota of milk have not been studied in depth in dairy cattle <sup>[18]</sup>, and not studied at all in sheep or goats. Thus, limited information is available on the microbiological quality of indoor air on dairy farms and the factors that influence it <sup>[20]</sup>, where the management of different substrates used to feed the animals and change the bedding, along with herd size and udder hygiene, may contribute to the transfer between the microorganisms present in the environment and the milk.

As regards the milking practice, which depends on the hygienic practices of the farmer during the process, the environmental sources of contamination may play an important role in the transfer of microorganisms, but this issue has not yet been carefully examined <sup>[18]</sup>. During and after milking, microbial contamination may occur through the different environmental sources, such as feeds, feces, bedding material, and soil, which adhere to the udder and from there, are transferred to the milk <sup>[21]</sup>. In view of the diversity of microbiota, we cannot discard the hypothesis that certain microorganisms present in the environment could be later found in the raw milk because of the animals housed in the stables. This transfer from the environment to the milk could take place either directly by air or through the teat, which has been in contact with various media (litter, dust, etc.), or because of the milking practice.

As previously mentioned, housing hygiene could have great importance in the control of dairy farm contamination, because it is considered an important source of microorganisms that could affect the quality of the milk <sup>[5]</sup>. Vissers et al. <sup>[22]</sup> found that the level of spore-forming butyric acid bacteria contamination was higher in cows that lay down on dirty patches of the housing, which resulted in highly contaminated teats before milking. This teat hygiene is related to the amount of dirt transmitted to the milk by a factor of 100 difference between a well-managed farm and badly-managed farm <sup>[23]</sup>. In addition, the hygiene of livestock can have an influence on the transmission of diseases. Rolesu et al. <sup>[24]</sup> showed that correct hygiene, with continuous removal of mud and manure from the livestock housing, drastically reduces the possibility of any outbreak of disease that could affect the animals.

As is the case with the dairy sector, environmental contamination in the cheese industry is of vital importance in the cheese-making process. Air is an important source of contamination, with microorganisms being transported through it, affecting the properties of the final product <sup>[25]</sup>. In addition, the hygienic quality of milk is very important, especially for milk that will be used for the elaboration of unpasteurized cheese <sup>[26]</sup>. For this reason, it is important to pay attention to possible routes of contamination through the dairy farm environment.

### **3. Effect of Temperature on Air Quality**

Temperature plays an important role in the environment of dairy farms because it is closely related to the welfare of the animals. In the coming years, due to climate change, the average global temperatures could rise by up to 4 °C in some parts of the world <sup>[27]</sup>. This increase will have an important impact on livestock farming systems, as well as on human and animal health <sup>[28]</sup>.

The effect of temperature on dairy farms depends on both the location and the season. In general, the different microclimatic conditions in each locality play a crucial role in the microbiological population, given that these microbial communities are very complex and variable to seasonal changes [29]. In this context, some researchers observed differences in the concentration of microorganisms in the environment, depending on the season [30] or due to meteorological factors [31]. As Sanz et al. [45] observed in their research of a dairy cattle farm, there was a seasonal effect on the number of isolates of bacteria (*Escherichia coli*) in the air, with up to twice the amount in the hot season (summer) than in the cold season (winter). Even the time of day influences the concentration of microorganisms. Popescu et al. [20] found a positive correlation between the increase in temperature and the increase in the mesophilic bacteria count in the environment, both in the morning and in the evening. For this reason, it is clear that seasonal and daily variations exist that make the bacterial counts higher in the hottest seasons as well as in the hottest hours of the day.

Environmental microorganisms are positively correlated with air temperature, but negatively correlated with humidity and solar radiation [31]. Regarding this fact, [32], in his research, concluded that if the study had been carried out in summer, the number of microorganisms detected in the air would probably have been higher. Calamari et al. [33], according to their research, noted a greater content of spores in feces during summer. These spores colonize the skin and the hair of animals and can contaminate the dairy farm environment. However, Adhikari et al. [34] found a higher concentration of spores and microorganisms in the environment in winter, indicating that it may be due to microclimatic differences between countries in which studies of this type are carried out, or that differences in diet composition might interfere with seasonal effects.

Good building design provides protection against climatic conditions (especially in areas which are more susceptible to extreme weather conditions), alleviating stress in the animals and avoiding increases in respiration rate and exchanges between the animal's body surface and the environment, which contribute to air pollution [35]. The choice of the adequate interval of temperature depends on the farming system. In dairy cattle, it is important to keep the animals between  $-5^{\circ}\text{C}$  and  $22^{\circ}\text{C}$  [36], although this thermal comfort zone varies depending on the physical condition of the animal, the resources available, and environmental factors. In the case of small ruminants, the optimum air temperature for efficient production should range between  $5^{\circ}\text{C}$  and  $20^{\circ}\text{C}$  [37].

In terms of animal welfare, some measures are strictly recommended depending on the season. During summer, sheep exposed to high temperatures and solar radiation show an increase in their respiration rate, an increase in their rectal temperature, and a metabolic alteration as opposed to those that stay in the shade (influencing the air quality), so the milk coagulating properties are diminished [38]. In addition, exposure to such radiation reduces the defensive capacity of the udder, which may allow contamination of the mammary gland by environmental pathogens [35]. Hence, providing shade in the barn during the hours of extreme heat minimizes the impact on the animal's body surface, improving the quality of the air and, thus, the quality of the milk [37]. During winter, the thermal comfort zone may vary depending on the physical and health conditions of the animal, the feeding, and other environmental factors. Therefore, the minimum thermal comfort value could be increased when the animals are wet or exposed to airflows or decreased when the relative humidity and solar radiation are high [39].

Airborne microbiota is also affected by the activities of the farm, and this could affect the environmental analysis developed. The best moment of the day to collect a sample or to measure certain parameters would be during the morning, before the aeration of the farm and before the beginning of farming activities, which contaminate the environment [20]. In addition, carrying out these analyses in the morning avoids the bactericidal effect of the radiation and helps to collect a wide range of airborne microorganisms [40].

The climate conditions also have an effect on the milking equipment. Poor hygiene in the milking parlor contributes to the pollution of the dairy farm environment and results in the contamination of the milking equipment. This situation, combined with high temperatures in the period between milking, increases the growth rate of microorganisms that affect the level of contamination in the milk [21]. This aspect is important when the milk is destined for use in the cheese-making process because the quality of the final product may be compromised.

## **4. Effect of Humidity on Air Quality**

A good livestock housing design is a fundamental factor to avoid the proliferation of microorganisms in the environment of dairy farms, and an essential aspect to be considered for the optimum project is humidity.

Relative humidity is important for health because it is one of the main factors that affect respiratory damage in both humans and animals. As Xiong et al. [41] said, the level of humidity is important for the welfare of animals because the

infectivity of pathogens found in the environment depends on this level.

It is important to take into account that the main parameters on the farm are interrelated: without good air distribution, poor ventilation will occur, the temperature will fluctuate from its optimum range, and relative humidity will be affected, influencing the count of microorganisms and molds. Moreover, as all environmental parameters are related in dairy farms, Sevi et al. [42] and Jimeno [43] concluded that certain space in the stables is necessary for each animal to ensure the correct relative humidity. If that living space is diminished, the concentration of pathogenic microorganisms in the environment is increased.

Dairy animals tolerate moisture poorly. The recommended values of relative humidity in dairy farms are between 55% and 75% for dairy cattle [36] and  $\leq 70\%$  for small ruminants [37]. However, these values overlap with the optimal level for the survival of most bacteria and fungi 55%–75% [41]. Moreover, Wilson et al. [40] found that low humidity negatively affects the collection of microorganisms from the environment, which may be due to their lower presence. For this reason, control of the humidity is necessary to minimize the risk.

Although Tang [44] found that the relationship between airborne bacteria with relative humidity is complicated, some researchers discovered that a positive correlation exists between the increased humidity of the air and fungi [20]. This indicates that fungi are the ones most affected by humidity. This is due to the fact that humid conditions induce the decomposition of organic materials in the livestock housing, which provides an excellent setting for the growth of fungi, and this contributes to an increase in the spore load in the farm environment [34]. In addition, it can be stated that there is a correlation between the concentrations of fungi in the environment and the different values of the relative humidity of indoor surfaces [45]. Therefore, it seems that there is a positive correlation between the humidity level of livestock housing and the fungi load in the environment.

## 5. Effect of Livestock Housing on Air Quality

The livestock housing has an important role in the dairy farm environment because a good design that promotes good health and welfare for the different species is a vital aspect of sustainable animal production, along with proper hygiene. The hygiene in the animal sheds of the farms includes air, bedding, and surface hygiene. Poor hygiene of surfaces is connected to intensive farming systems, and is aggravated by poor maintenance [46].

Microbial communities present in livestock housing are interrelated with those present in the milking parlor. Good milking practice is another factor in a proper dairy farm environment because the correct management of the milking parlor improves the quality of the milk [18]. Hence, good hygiene among the farm-workers during milking (cleaning of the machines, hygienic parlor, udder disinfection, etc.) affects the concentration of microorganisms in the environment, both those bacteria which are favorable to the quality of the milk and those which are harmful to this quality [47]. Generally, increasing the times between two milking intervals provides the microorganism with more time to grow, increasing the microbial concentration in the environment that surrounds the milking equipment, and affecting the level of contamination in the milk [21]. In addition, poor hygiene has negative effects on milk yield, causing low concentrations of protein and fat; and an increase in somatic cells in the animals of the herd, which in turn decreases the quality of the milk [9].

It is required that the dairy farm be well designed. Proper air space for the animals is one of the most important factors for the quality of the air and the welfare of the animals in the livestock houses. Air quality in the livestock building is a source of contamination not only for the animals but also for the workers' health. The optimal volume to avoid an increase in the total mesophilic bacteria count and the relative humidity in the environment, as well as a decrease in the properties of the milk, is 35–40 m<sup>3</sup>/head for cattle [48] and 7 m<sup>3</sup>/head for sheep and goats [38].

Another factor that affects air quality in livestock is the stocking density. The concentration of microorganisms and particles in the environment in livestock housing is inversely related to space. Concentrations of total mesophilic bacteria in the livestock house is significantly lower when animals have a stocked density of 5 m<sup>2</sup>/head for cows [43] and 2 m<sup>2</sup>/head for sheep or goats, and the somatic cell count in small ruminants is up to four times lower than animals stocked at 1–1.5 m<sup>2</sup>/head [42].

Furthermore, the material used in livestock housing affects the concentration of microorganisms in the air. If there is hay on the floor of the housing, it may increase the propionic bacteria and *Lactobacillus* [49]; the use of sand increases the number of *Streptococcus*, and the use of sawdust increases the level of coliforms and *Klebsiella* in the animals [50]. However, Monsallier et al. [51] showed that the use of straw as a bedding material, as opposed to other materials, means an increase in the microbial levels of the herds, which implies a higher level of ripening bacteria in the milk.

The material that covers the floor of the house is a source of contamination. The concentration of microorganisms in the dairy farm environment tends to be higher in those farms where the bedding is not clean or is not changed frequently [20][52]. Therefore, it is necessary to change the bedding material daily or add another layer of material each day to reduce the microbial load significantly. It has been demonstrated that fresh bedding usually has much lower microbial concentrations than used bedding because the concentration of microorganisms tends to increase due to contamination from feces and microbial growth during the first day [53]. Treating the bedding with products that reduce microbial activity (such as bentonite) can be a good strategy to reduce contamination in the livestock environment [46]. Bentonite is a volcanic material composed mainly of clay minerals and has a great capacity for water absorption. The application of bentonite to the bedding achieves a significant reduction in the number of microorganisms in the environment, which improves the quality of the milk [52]. It can also partially alleviate the adverse effects of the stocking density in farms, improving the coagulation capacity and, thus, the quality of the milk [54]. Therefore, good maintenance of bedding on farms is a critical point for livestock hygiene. It is clear that there is a transfer of microorganisms between the environment and the milk, and it is for this reason that good health and hygienic parameters are very important in the production of high-quality milk.

Apart from those factors already mentioned, another significant parameter is the feed. Diet may play an important role in the dairy farm environment because the air quality is influenced by the feces of the animals, which is an important contamination factor. It is well known that feed introduces a large variety of microorganisms and spores into the environment and, therefore, into the milk [21]. This can be seen in the results of Calamari et al. [33], where the concentration of spores in feces, and consequently, the contamination of the environment, is strictly related to the content of spores in the feed. It is important to control the impact that the feed has on the dairy farm environment because it not only affects the quality of the milk but also acts as a disseminator of pathogens affecting the animals on the farm.

Despite the high number of microorganisms present in the air in the dairy farm environment, only a third of them are detected in the milk [18], which means that there is a partially efficient (but not impervious) barrier between the barn and the milk. The effectiveness of this barrier is influenced by the hygienic conditions of the farm. The better the hygiene in livestock house, the lower the count of total mesophilic bacteria and the higher the quality of the milk. In this regard, Oliete et al. [55] demonstrated with ovine that the quality of the milk is related to good hygienic practices on the farm. In a similar case studied by Bergonier et al. [56], a relation was found between the poor hygiene of the milking parlor and the livestock house and the increase in total mesophilic bacteria, and, thus, poor milk quality.

As previously mentioned, the environment of livestock housing has important effects on the quality of milk. This implies that the quality of dairy products, particularly in the cheese-making industry, could be affected too. It is essential to consider the correct parameters in animal welfare, which have an influence on dairy products. As Sevi et al. [42] demonstrated, the milk from ewes stocked at 2 m<sup>2</sup>/head showed a significant increase in milk coagulating properties, avoiding harmful bacteria in the environment and enhancing cheese-making bacteria crucial for the manufacturing process. In addition, Albenzio et al. [57] discovered that the control of housing sanitation and milking procedures is critical to obtain good quality milk and its products. Meanwhile, Monsallier et al. [51] demonstrated the importance of bedding material as a source of beneficial bacteria in ripening for cheese making, with the type of bedding material used for the stables acquiring major significance.

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